Current FDA approach for preclinical vector biodistribution studies

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Background

- **Origin of concern**
  - **Preclinical data**
    - **unexpected persistence in gonads**
    - **inadequacy of preclinical data**
  - **Vector integration**
    - **literature examples of plasmid DNA integration**

    (vector least expected to be of concern)
- **PCR signal in gonads not necessarily in germ cells nor integrated**

Plasmid integration-1

- **B lymphocytes**
  - **In vivo role of B lymphocytes in somatic transgene immunization. Xiong et al 1997. PNAS 94: 6352**
    - **100µg DNA intrasplenic**
    - **persistence 3 months**
    - **integration in B cells (digest:self-ligate:PCR)**

Plasmid Integration-2

- **Lung and kidney**
  - **Safety study and characterization of E1A-liposome complex gene delivery protocol in an ovarian cancer model. Xing et al. 1998. Gene Therapy 5 : 1538**
    - **– plasmid:liposome intraperitoneal**
    - **– plasmid DNA in lung and kidney 1 1/2 year post administration: not in ovaries**
      - **» integrated (DpnI digestion)**

Assay Purpose and Selection

- **Distribution studies are designed to address two issues:**
  - **i) potential for germline alteration**
  - **ii) potential for toxicity in other tissues and organs**

    (as for other biological products)
- **Detection method should be suitable for clinical samples as well as in animal samples**

In vivo Treatment

- **male and/or female animals**
  - **appropriate to clinical use, potential future clinical use**
    - **age**
    - **developmental state**
    - **group size**
- **vector administered by clinical route**
- **dose selection**
    - **vehicle control**
    - **clinically relevant dose**
    - **exceed clinically relevant dose**

Sacrifice Times

- **early - peak vector levels**

- **multiple time points to assess duration of vector persistence**

Tissue Harvest

- **peripheral blood, injection site, gonads (other organs as needed for more general toxicity studies)**
- **harvest using procedures to minimize cross contamination**

Assay Parameters

- **assay significant sample of genomic DNA from each tissue**
- **use spike controls**
- **perform replicate tests**
- **adequate PCR sensitivity**

PCR Sensitivity and Sample Size Influences Risk Assessment

- **Sensitivity**
  - **capable of 1 copy/μg genomic DNA**
- **Sample size**
  - **sufficient replicates**
- **Statistical Considerations**

PCR product detection methods

- **Southern**
  - **shows size and specificity**
- **Slot or dot blot**
  - **probe indicates specificity**
- **Real time**
  - **newest technology, kinetic analysis needed**
- **All capable of 1 copy/μg**

Primer Selection

- **Detection of therapeutic gene not necessary to address germline insertion**

- **Choose unique amplicon**

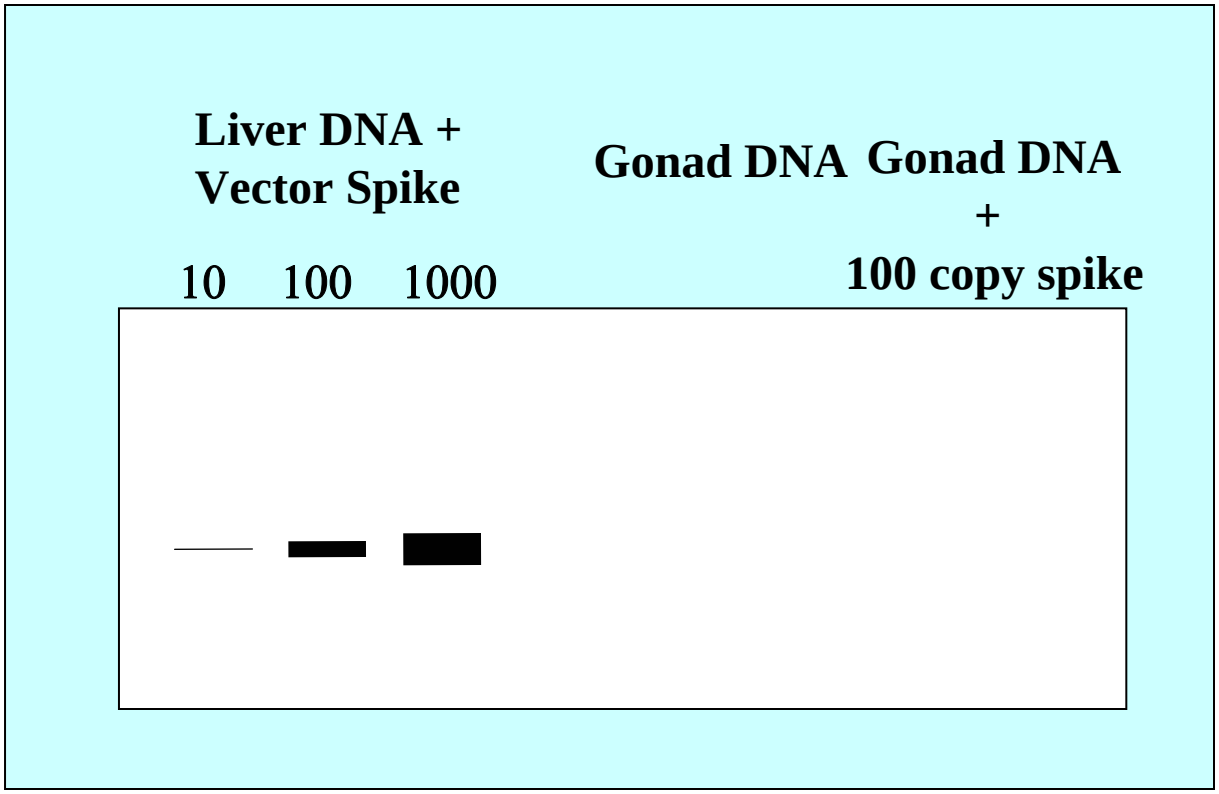
Minimal PCR Recommendations

- **3 samples/tissue, 1 μg genomic DNA each**
  (or sufficient replicates to total sampling of 3 μg)
- **2 samples run unspiked, 1 sample/tissue run with spiked control**
- **sensitivity <100 copies of vector/1 μg genomic DNA**
- **assay should be adequate for study of clinical samples as well as in animal tissues**

Technical Considerations

- **Spiking in gonadal tissue**
  - **sensitivity control**
    - **signal extinction**
      - **contaminants, competition**
    - **purification control**
      - **check for loss of vector during DNA purification**

Signal Extinction



PCR, gel electrophoresis

Impact on Informed Consent

- **statement in informed consent regarding current results, unknown risk of vector dissemination and transmission to germ cells**
- **temporary use of contraception recommended**
- **autopsy requested in treated patients**

Impact of Results on Clinical Development

- **gonadal signal**
  - **if assay is adequate**
    - **not detected at all times**
    - **transiently positive**
    - **persistently positive**

Gonadal Signal Undetectable at all Time Points

- **clinical study may proceed, no restrictions on patient population**

Gonadal Signal Transiently Positive

- **re-evaluate clinical study as to patient population, severity of illness; may proceed if benefit justifies risk**
- **semen analysis in treated males requested in follow-up where applicable/appropriate**

Gonadal Signal Persistently Positive

- restrict patient population to sterile individuals
- semen analysis in treated males requested in follow-up where applicable/appropriate
- analyze source of signal

Persistent Signal Source

- **Contamination**
- **Signal not from contamination**
  - **determine duration of positive signal**
  - **determine cellular source**

Acknowledgements

- **FDA appreciates efforts of the RAC and NIH to support public discussion of these issues and to encourage research to answer these questions**

Primer selection

- **Detection of therapeutic gene not necessary to address germline insertion**
- **Choose unique amplicon**
  - **amplify from gene to vector backbone or vector only**
  - **avoid:**
    - **homology with endogenous gene**
    - **multigene families**

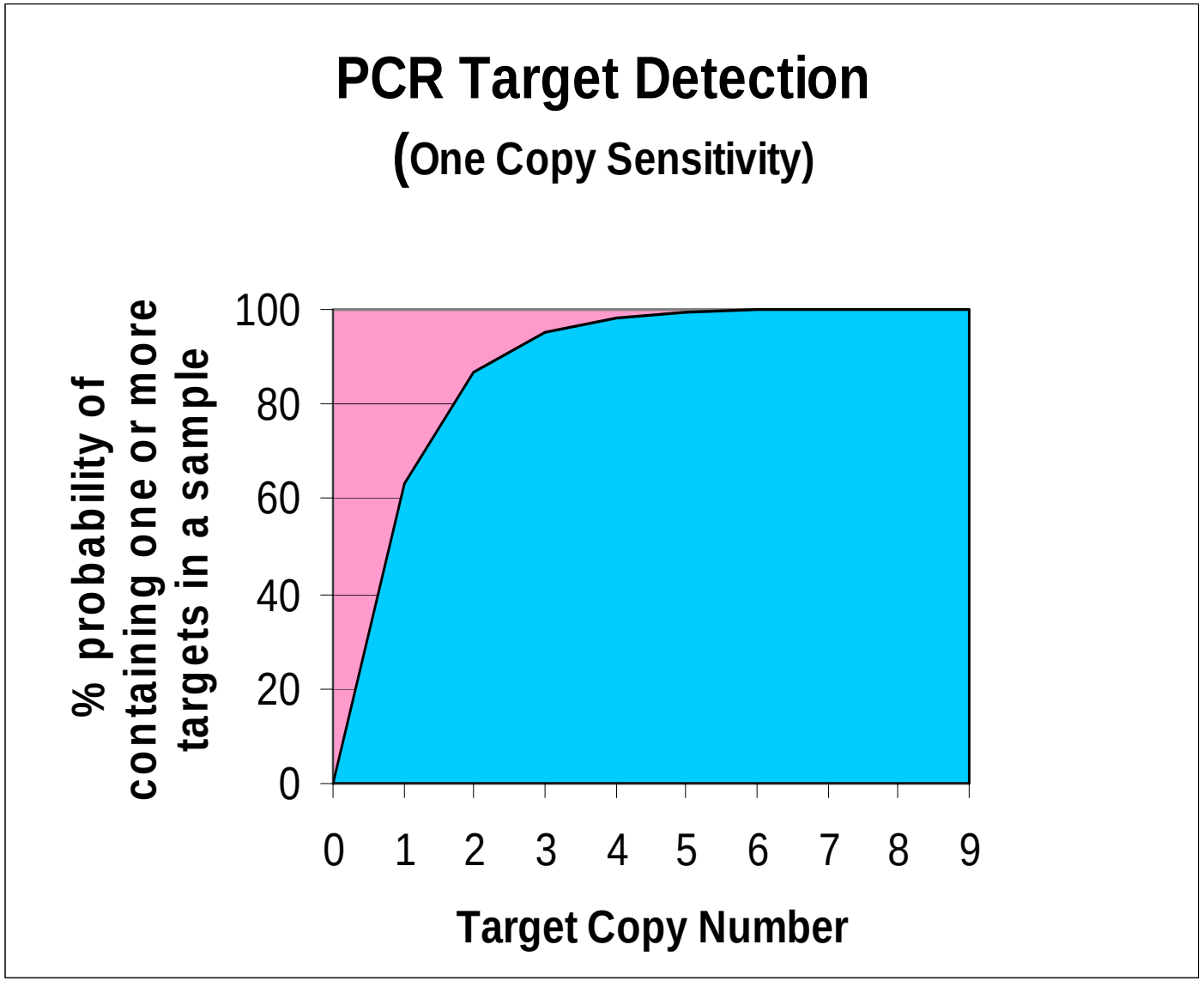
Determination of Signal Source

- **Contamination**
  - **during harvest**
    - **re-evaluate procedures for tissue harvest**
    - **sterile instruments, order of organs procured**
  - **from prior PCR**
    - **utilize PCR “sterilization” techniques, UDG**
    - **evaluate with other primer sets**

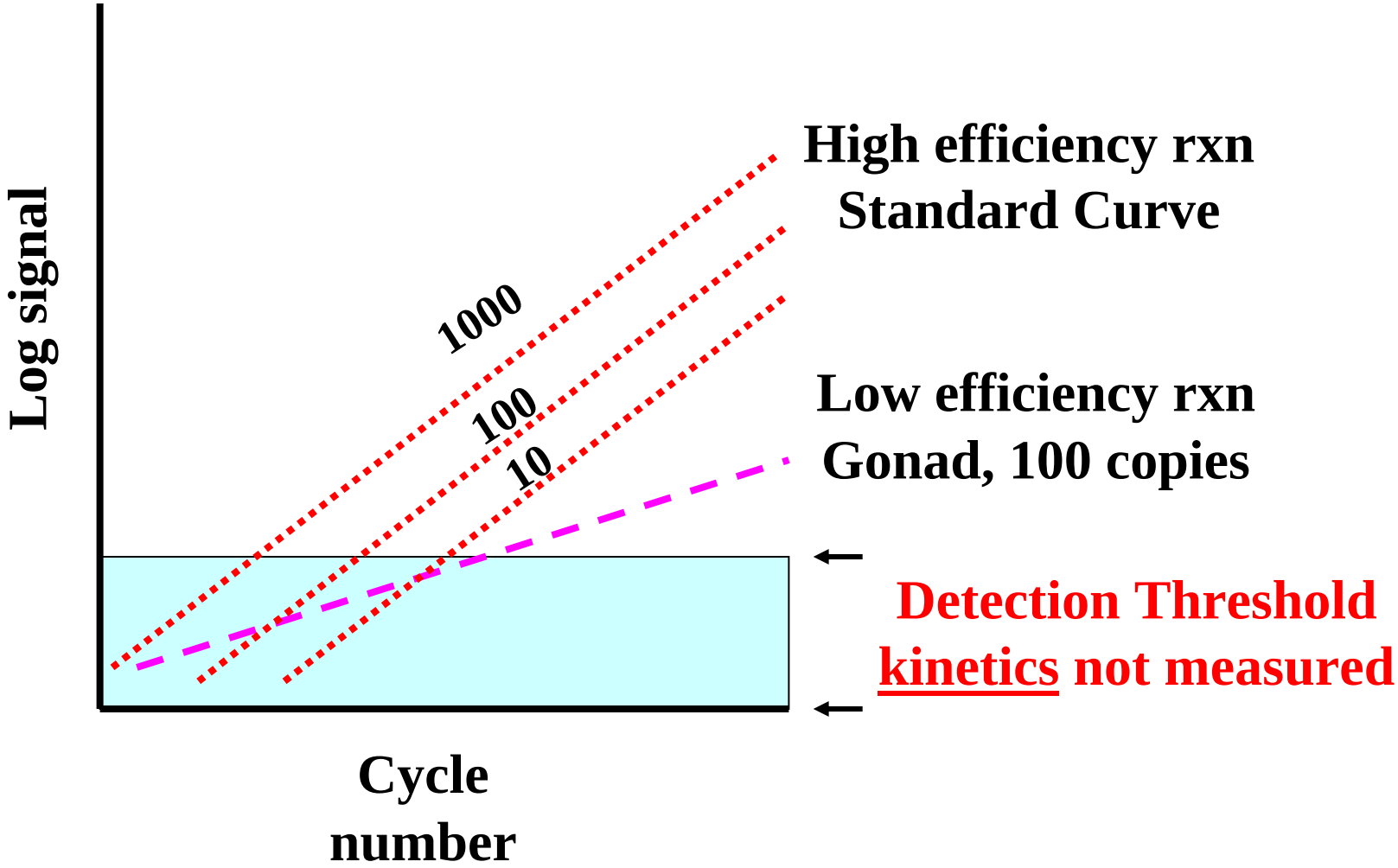
Signal Source

- **Signal not from contamination**
  - **determine duration of positive signal**
  - **determine cellular source**
    - **analyze semen, sperm**
    - **in situ PCR**
    - **other novel techniques and approaches**
      - **platform studies**

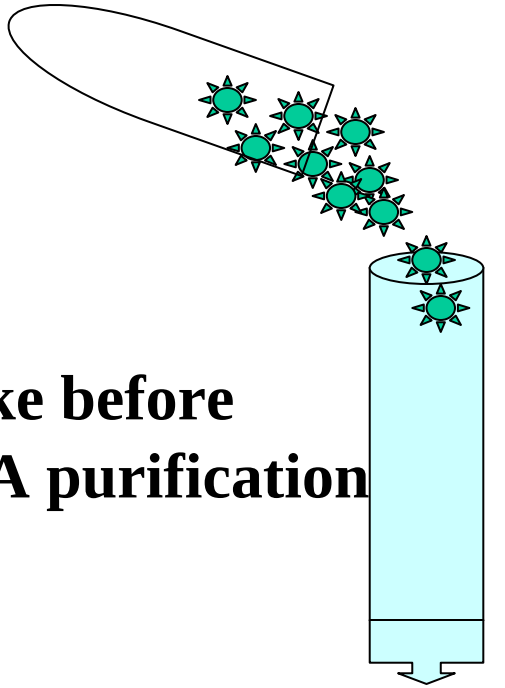
Statistical Considerations



Real Time PCR

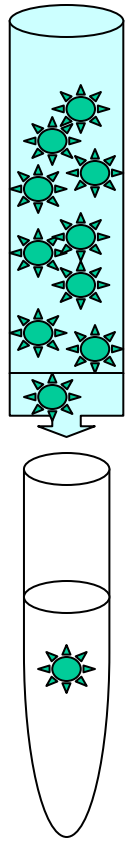


Vector/DNA Recovery



Spike before DNA purification

elution



Determine recovery efficiency